

CARBOHYDRATE RESEARCH

Carbohydrate Research 305 (1998) 289-292

Note

Use of a phenyl 1-selenogalactofuranoside as a glycosyl donor for the synthesis of galactofuranosyl-containing disaccharides

Blair D. Johnston, B. Mario Pinto *

Department of Chemistry and Institute of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6

Received 9 May 1997; accepted in revised form 5 August 1997

Abstract

The use of acetylated phenyl 1-seleno- β -D-galactofuranoside as a glycosyl donor for the synthesis of protected D-Galf- β -(1 \rightarrow 3)- α -D-Manp as its methyl or ethylthio glycoside has been demonstrated. Activation of the selenoglycoside over a thioglycoside acceptor by NIS/TfOH is extremely selective and gives the ethylthio disaccharide in 91% yield. The parent disaccharide is found as a terminal and branched unit in the lipopeptidophosphoglycan oligosaccharides of the protozoan *Trypanosoma cruzi*, the causative agent of Chagas' disease. © 1998 Elsevier Science Ltd

Keywords: β -D-galactofuranose: Selenoglycosides; Thioglycosides; Disaccharides; Galactofuranosyl; *N*-iodosuccinimide; Selective selenoglycoside activation; *Trypanosoma cruzi*

1. Introduction

The presence of galactofuranosyl units in polysaccharides is restricted to lower organisms such as bacteria [1], protozoa [2] or fungi [3]. The potential for taking advantage of this anomaly in drug therapy or immunological applications is a topic of current investigation. Oligosaccharide synthesis involving furanosyl glycosyl donors has been studied only sparingly compared to pyranosyl donors, but methods have been demonstrated for thioglycosides [4], *n*pentenyl glycosides [5], anomeric benzoates [6] and anomeric xanthates [7]. Most recently, McAuliffe and Hindsgaul [8] have described an indirect approach to galactofuranosyl-containing disaccharides involving acyclic glycosyl donors. A recent communication regarding the use of phenyl 1-selenoribofuranosides as furanosyl donors [9] has prompted us to report our preliminary findings using a phenyl selenogalactofuranosyl donor.

The lipopeptidophosphoglycan (LPPG) oligosaccharides of *Trypanosoma cruzi*, the protozoan responsible for Chagas' disease, include $O-\beta$ -Dgalactofuranosyl- $(1 \rightarrow 3)$ - α -D-mannopyranosyl disaccharides as terminal or branched components [10]. As an extension of our previous studies of the selective activation of selenoglycoside glycosyl donors in the presence of thioglycoside glycosyl acceptors [11], we now report the synthesis of phenyl 2,3,5,6-tetra-Oacetyl- β -D-selenogalactofuranoside (**2**) and its use as a galactofuranosyl donor for glycosylation of the

^{*} Corresponding author. Tel.: +1-604-291-4327; fax: +1-604-291-3765; e-mail: bpinto@sfu.ca.

3-OH position of methyl- and ethylthio- α -D-mannopyranosides 3 and 5.

Reaction of 1, 2, 3, 5, 6-penta-O-acetyl- β -D-galactofuranose [12] with benzeneselenol and boron trifluoride-etherate [11] gave a virtually quantitative yield of the phenyl β -D-selenofuranoside (2). The anomeric configuration of 2 was confirmed by a ^{1}H NMR NOESY spectrum which showed substantial H-1/H-3 and H-1/H-5 NOE contacts and a lack of such a contact between H-1 and H-4. The H-1 resonance in the ¹H NMR spectrum of **2** appeared as a closely spaced multiplet at δ 5.77 instead of the expected doublet. This was proven to be the result of the long-range coupling of H-1 with H-3 and H-4, as evidenced by the presence of weak correlations of H-1 with both H-3 and H-4, in addition to the strong er H-1/H-2 cross-peaks, in the COSY spectrum of 2. There was no evidence by ¹H NMR for the presence of more than 5% of the α -anomer in the crude reaction mixture. Purification by chromatography gave 2 as a syrup, which could not be induced to crystallize, but which was homogeneous by TLC and NMR, and is stable for months in the freezer.

Reaction of the furanosyl donor 2 with methyl 2-O-benzyl-4,6-O-benzylidene- α -D-mannopyranoside (3) [13] using our previous conditions [11] for the activation of selenoglycosides (excess AgOTf/potassium carbonate) was unsuccessful due to decomposition of the acid-sensitive furanoside product. The alternative promoter system *N*-iodosuccinimide/triflic acid (NIS/TfOH) developed by Zuurmond et al. [14] was more suitable in this case and reaction at room temperature (TfOH:NIS:2:3 = 0.04:1:1:1) gave disaccharide 4 (69%) which was isolated as a crystalline solid.

The reaction of an excess of 2 with ethyl 2-O-benzoyl-4,6-O-benzylidene-1-thio- α -D-mannopyranoside (5) [15] at room temperature, with the assistance of NIS/TfOH was extremely rapid, and gave an excellent yield of disaccharide 6 (91% based on 5). A similar reaction without inclusion of a catalytic amount of triflic acid demonstrated that, although NIS alone will activate selenoglycosides, the major product in this case was the sensitive orthoester derivative 7 which was isolated in only moderate yield due to hydrolysis during processing and chromatography. We have occasionally isolated orthoester derivatives from selenoglycoside glycosylations and implicated them as intermediates by showing their Lewis-acid catalyzed rearrangement to glycosides [11]. The disaccharides 4 and 6 and the orthoester 7 exhibited unexceptional NMR spectra which were assigned completely through application of two-dimensional techniques.

In summary, the selenoglycoside 2 has been synthesized and shown to have potential as a versatile galactofuranosyl glycosyl donor. The selective activation of a selenofuranoside in the presence of a thiopyranoside using NIS/TFOH has been demonstrated and applied to the synthesis of a disaccharide 6 that may be used directly as a glycosyl donor for the elaboration of higher-order oligosaccharides. Attachment of an appropriate linker-arm can also lead to the synthesis of glycoconjugates [16]. Compound 6 is a protected form of the naturally occurring disaccharide moiety in the LPPG of *T. cruzi*. Further applications of 2 and 6 in the preparation of other galactofuranosyl oligosaccharides of biological importance are underway.

2. Experimental

General methods.—TLC was performed using aluminum plates, precoated with Merck Silica Gel $60F_{254}$, using appropriate mixtures of hexanes–EtOAc or toluene–EtOAc for development. Visualization was achieved by exposure of the dried plates to UV light or by spraying with a solution of 1% ceric sulfate and 1.5% molybdic acid in 10% aqueous H₂SO₄ and heating. Column chromatography was performed using Silica Gel 60 (Merck, 230–400 mesh).

Optical rotations were measured at 21 °C with a Rudolph Research Autopol II polarimeter. Melting points were determined with a Fisher–Johns melting point apparatus and are uncorrected. ¹H and ¹³C NMR spectra were obtained using a Bruker AMX-400 spectrometer operating at 400.13 and 100.6 MHz for proton and carbon respectively. Chemical shifts in CDCl₃ are reported relative to external TMS. All assignments were confirmed with the aid of two-dimensional ¹H/¹H (COSYDFTP) or ¹H/¹³C (IN-VBTP) experiments using standard Bruker pulse programs and an inverse-detection, ¹H/X double-resonance probe. Chemical shifts and coupling constants were obtained from a first-order analysis of the spectra.

Phenyl 2, 3, 5, 6 - *tetra* - O - *acetyl* - *l* - *seleno* - β - D galactofuranoside (2).—A solution of 1,2,3,5,6penta-O-acetyl-β-D-galactofuranose [12] (7.81 g, 20.0 mmol) and benzeneselenol (4.0 g, 25 mmol) in CH₂Cl₂ (70 mL) was stirred with boron trifloride:etherate (2.5 mL, 20 mmol) at room temperature for 75 min. The mixture was diluted with CH₂Cl₂ (100 mL) poured into a mixture of

ice/saturated NaHCO₃ (\sim 75 mL each) and stirred until the bubbling stopped. The aqueous phase was extracted with more CH₂Cl₂ (2×30 mL) and the combined extracts were washed with saturated aqueous NaHCO₃ solution (30 mL) and water (30 mL), dried over anhydrous MgSO₄ and concentrated to give the crude selenoglycoside (2) as a yellow oil. Chromatography on silica gel (2:1 hexanes-EtOAc) gave pure 2 as a very pale-yellow syrup (9.64 g, 99%); $[\alpha]_{\rm D} = -152^{\circ} (c \ 1.0, \ \text{CHCl}_3);$ ¹H NMR: δ 7.62-7.58 (m, 2 H, aromatic), 7.33-7.27 (m, 3 H, aromatic), 5.78 (m, 1 H, $J_{1,2}$ 1.6, $J_{1,3}$ 0.8, $J_{1,4}$ 0.6 Hz, H-1), 5.43 (ddd, 1 H, $J_{4,5}$ 4.0, $J_{5,6a}$ 4.5, $J_{5,6b}$ 7.1 Hz, H-5), 5.30 (dd, 1 H, J_{2.3} 3.6 Hz, H-2), 5.06 (ddd, 1 H, $J_{3,4}$ 5.6 Hz, H-3), 4.48 (ddd, 1 H, H-4), 4.31 (dd, 1 H, J_{6a.6b} 11.8 Hz, H-6a), 4.17 (dd, 1 H, H-6b), 2.12, 2.11, 2.09, 2.04 (4 s, each 3 H, 4 $COCH_3$); ¹³C NMR: δ 170.44, 169.97, 169.79, 169.54 (4 COCH₃), 134.51(2 C), 129.13 (2 C), 128.77, 128.13 (Ar), 86.30 (C-1), 82.07 (C-2), 80.61 (C-4), 76.52 (C-3), 69.01 (C-5), 62.54 (C-6), 20.76, 20.70, 20.67 (2 C), (4 COCH₃). Anal. Calcd for $C_{20}H_{24}O_9$ Se: C, 49.29, H, 4.96. Found: C, 49.11; H, 4.72.

Methyl 2-O-benzyl-4,6-O-benzylidene-3-O-(2,3,5,6tetra - O - acetyl - β - D - galactofuranosyl) - α - D mannopyranoside (5).—The selenoglycoside 2 (120) mg, 0.246 mmol) and the selectively protected mannopyranoside 3 [13] (89 mg, 0.24 mmol) were dissolved in a mixture of CH₂Cl₂ (5 mL) and Et₂O (1 mL). The solution was stirred with freshly activated powdered 4 A molecular sieves (0.5 g) under a nitrogen atmosphere at room temperature for 15 min. N-iodosuccinimide (56 mg, 0.25 mmol) was added followed, after 5 min, by triflic acid (1 μ L, 0.01 mmol) via syringe. An immediate reaction to produce a dark purple-brown color ensued. After 40 min, triethylamine (0.020 mL) was added to quench the reaction and the mixture was filtered through Celite with the aid of additional CH_2Cl_2 (50 mL). The filtrate was washed with 10% aqueous NaS₂O₃ (10 mL) and saturated NaHCO₃ (15 mL), dried (MgSO₄), and concentrated to give a yellow syrup. TLC (3:2 hexanes-EtOAc) indicated that 2 had been completely consumed and that a slightly more polar product had been formed. Chromatography on silica gel (3:2 hexanes-EtOAc) gave 4 as a colorless solid (116 mg, 69%). An analytically pure sample was obtained by crystallization from CH₂Cl₂/hexanes: mp 160–161 °C; $[\alpha]_{\rm D} = 24^{\circ} (c \ 0.5, \ \text{CHCl}_3);^{-1}\text{H}$ NMR: δ 7.50-7.27 (m, 10 H, aromatic), 5.59 (s, 1 H, PhC H), 5.29 (ddd, 1 H, $J_{4',5'}$ 3.4, $J_{5',6a'}$ 7.6, $J_{5',6b'}$ 4.1 Hz, H-5'), 5.07 (s, 1 H, H-1'), 5.04 (d, 1 H, $J_{2'3'}$

1.4 Hz, H-2'), 4.91 (dd, 1 H, $J_{3',4'}$ 5.8 Hz, H-3'), 4.85, 4.72 (2d, each 1 H, $J_{a,b}$ 12.2 Hz, PhC H_2 O), 4.70 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.36 (ddd, 1 H, $J_{4',5'}$ 3.4 Hz, H-4'), 4.26 (dd, 1 H, $J_{5.6a}$ 4.5, $J_{6a.6b}$ 9.9 Hz, H-6a), 4.18 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 10.1 Hz, H-3), 4.10 (dd, 1 H, $J_{4,5}$ 8.9 Hz, H-4), 4.09 (dd, 1 H, $J_{6a',6b'}$ 11.9 Hz, H-6a'), 3.91 (dd, 1 H, H-6b'), 3.86 (dd, 1 H, J_{5.6b} 10.1 Hz, H-6b), 3.82–3.74 (m, 2 H, H-2, H-5), 3.34 (s, 3 H, OC H₃), 2.11, 2.08, 1.93, 1.91 (4 s, each 3 H, 4 COC*H*₃); ¹³C NMR: δ 170.39, 170.05, 169.98, 169.73, (4 COCH₃), 138.07, 137.69, 128.97, 128.41 (2 C), 128.18 (2 C), 128.03 (2 C), 127.78, 126.02 (2 C) (12 C aromatic), 102.27 (C-1'), 101.70 (PhCH), 100.52 (C-1), 81.66 (C-2'), 80.06 (C-4'), 76.85 (C-4), 76.75 (C-3'), 74.86 (C-2), 73.91 (PhCH2), 71.88 (C-3), 69.28 (C-5'), 68.89 (C-6), 64.20 (C-5), 62.83 (C-6'), 54.90 (OCH₃), 20.78 (2 C), 20.57, 20.47 (4 COCH₃). Anal. Calcd for $C_{35}H_{42}O_{15}$: C, 59.82; H, 6.02. Found: C, 59.88; H, 5.98.

Ethyl 2-O-benzoyl-4,6-O-benzylidene-3-O-(2,3,5,6tetra-O-acetyl- β -D-galactofuranosyl)-1-thio- α -Dmannopyranoside (6).—The selenoglycoside 2 (223 mg, 0.458 mmol) and the selectively protected ethylthio mannopyranoside **4** [15] (106 mg, 0.254 mmol) were dissolved in a mixture of CH_2Cl_2 (7 mL) and Et_2O (1.5 mL). The solution was stirred with freshly activated powdered 4 Å molecular sieves (0.5 g)under a nitrogen atmosphere at room temperature for 15 min. N-iodosuccinimide (104 mg, 0.462 mmol) was added followed, after 5 min, by triflic acid (2 μ L, 0.02 mmol) via syringe. An immediate reaction to produce a dark purple-brown color ensued. After 15 min, triethylamine (0.050 mL) was added to quench the reaction and the mixture was filtered through Celite with the aid of additional CH₂Cl₂ (100 mL). The filtrate was washed with 10% aq NaS_2O_3 (10 mL) and water (10 mL), dried (MgSO₄), and concentrated to give an orange oil. TLC (3:1 toluene-EtOAc) indicated that 2 had been completely consumed and that a slightly more polar product had been formed. Chromatography (3:1 toluene-EtOAc) gave **6** as a colorless foam (172 mg, 91%): $[\alpha]_{\rm D} = 5^{\circ}$ $(c \ 0.6, \text{CHCl}_3);$ ¹H NMR: $\delta \ 8.12-7.32$ (m, 10 H, aromatic), 5.64 (dd, 1 H, J_{1.2} 1.1, J_{2,3} 3.5 Hz, H-2), 5.63 (s, 1 H, PhCH), 5.41 (d, 1 H, H-1), 5.31 (ddd, 1 H, $J_{4',5'}$ 3.1, $J_{5',6a'}$ 7.1, $J_{5',6b'}$ 4.7 Hz, H-5'), 5.26 (s, 1 H, H-1'), 4.97 (d, 1 H, $J_{2',3'}$ 1.9 Hz, H-2'), 4.86 (dd, 1 H, $J_{3',4'}$ 5.8 Hz, H-3'), 4.34 (ddd, 1 H, $J_{4,5}$ 9.7, J_{5.6a} 4.8, J_{5.6b} 10.0 Hz, H-5), 4.33 (dd, 1 H, J_{3.4} 10.0 Hz, H-3), 4.28 (dd, 1 H, $J_{6a,6b}$ 10.0 Hz, H-6a), 4.27 (dd, 1 H, H-4'), 4.12 (dd, 1 H, $J_{6a'.6b'}$ 12.0 Hz, H-6a'), 4.08 (dd, 1 H, H-4), 4.07 (dd, 1 H, H-6b'),

3.92 (dd, 1 H, H-6b), 2.75–2.60 (m, 2 H, SC H_2 CH₃), 2.09, 2.07, 1.93, 1.81 (4 s, each 3 H, 4 COC H_3), 1.32 (t, 3 H, J 7.4 Hz, SCH₂C H_3); ¹³C NMR: δ 170.37, 169.95, 169.87, 169.14 (4 COCH₃), 165.52 (COPh), 137.41, 133.37, 129.94 (2 C), 129.71, 129.10, 128.48 (2 C), 128.25 (2 C), 126.02 (2 C) (12 C aromatic), 102.33 (C-1'), 101.82 (PhCH), 83.48 (C-1), 80.83 (C-2'), 80.29 (C-4'), 77.43 (C-4), 76.84 (C-3'), 70.84 (C-2), 70.00 (C-3), 69.14 (C-5'), 68.70 (C-6), 64.65 (C-5), 62.68 (C-6'), 25.62 (SCH₂CH₃), 20.76, 20.70, 20.61, 20.29 (4 COCH₃), 14.87 (SCH₂CH₃). Anal. Calcd for C₃₆H₄₂O₁₅S: C, 57.90; H, 5.67. Found: C, 57.97; H, 5.73.

3,5,6-Tri-O-acetyl-1,2-(ethyl 2-O-benzoyl-4,6-Obenzylidene - 1 - thio - α - D - mannopyranos - 3 - yl) - α - D galactofuranose orthoacetate (7).—The selenoglycoside 2 (150 mg, 0.308 mmol) and the selectively protected ethylthio mannopyranoside 4 (102 mg, 0.245 mmol) were dissolved in a mixture of CH₂Cl₂ (7 mL) and Et₂O (1.5 mL). The solution was stirred with freshly activated powdered 4 Å molecular sieves (0.5 g) under a nitrogen atmosphere at room temperature for 15 min. N-iodosuccinimide (70 mg, 0.31 mmol) was added. A slow reaction to produce a purple color ensued. After 7 h, TLC (3:1 toluene-EtOAc,) indicated that 2 had been consumed and a less polar component had formed. The reaction mixture was processed as described in the preparation of 6. Purification by chromatography on silica gel (3:1 toluene-EtOAc) gave 7 as a colorless foam (102 mg, 56%). Significant hydrolysis of the orthoester 7 had occurred during processing and chromatography to regenerate 4 (33 mg) which was also isolated. Compound 7 was isolated as a pure diastereomer: $[\alpha]_{D}$ $+44^{\circ}$ (c 0.6, CHCl₃); ¹H NMR: δ 8.12–7.32 (m, 10 H, aromatic), 5.84 (d, 1 H, J_{1'.2'} 4.3 Hz, H-1'), 5.61 (s, 1 H, PhCH), 5.52 (d, 1 H, J_{1,2} 1.4, J_{2,3} 3.4 Hz, H-2), 5.33 (d, 1 H, H-1), 5.18 (ddd, 1 H, $J_{4'5'}$ 9.3, $J_{5',6a'}$ 3.7, $J_{5',6b'}$ 5.5 Hz, H-5'), 5.08 (d, 1 H, $J_{2',3'}$ ~ 0, $J_{3',4'}$ 1.3 Hz, H-3'), 4.82 (d, 1 H, H-2'), 4.41 (dd, 1 H, $J_{6a',6b'}$ 12.4 Hz, H-6a'), 4.33 (ddd, 1 H, $J_{4,5}$ 9.8, $J_{5,6a}$ 5.0, $J_{5,6b}$ 10.1 Hz, H-5), 4.28 (dd, 1 H, $J_{3,4}$ 10.1 Hz, H-3), 4.27 (dd, 1 H, J_{6a.6b} 10.1 Hz, H-6a), 4.15 (dd, 1 H, H-4'), 4.08 (dd, 1 H, H-6b'), 4.07 (dd, 1 H, H-4), 3.90 (t, 1 H, H-6b), 2.75–2.60 (m, 2 H, SCH_2CH_3 , 2.07, 2.06, 2.03 (3 s, each 3 H, 3 $COCH_3$), 1.72 (s, 3 H, orthoester CH_3), 1.32 (t, 3 H, J 7.4 Hz, SCH₂CH₃); ¹³C NMR: δ 170.49, 169.92, 169.59 (3 COCH₃), 165.68 (COPh), 137.25, 133.46, 129.94 (2 C), 129.58, 129.04, 128.51 (2 C), 128.24 (2 C), 126.04 (2 C) (12 C aromatic), 123.97 (orthoester C), 106.04 (C-1'), 102.04 (PhCH), 84.83 (C-4'), 84.32 (C-2'), 83.61 (C-1), 77.83 (C-4), 76.40 (C-3'),

74.03 (C-2), 69.99 (C-5'), 68.94 (C-3), 68.67 (C-6), 65.01 (C-5), 62.83 (C-6'), 25.62 (SCH₂CH₃), 22.10, 20.86, 20.67 (2 C) (3 COCH₃, orthoester CCH3), 14.89 (SCH₂CH₃). Anal. Calcd for $C_{36}H_{42}O_{15}S$: C, 57.90; H, 5.67. Found: C, 57.81; H, 5.62.

Acknowledgements

We are grateful to the Natural Sciences and Engineering Research Council of Canada for financial support.

References

- M. McNeil, S.J. Wallner, S.W. Hunter, and P.J. Brennan *Carbohydr. Res.*, 166 (1987) 299–308.
- [2] R.M. de Lederkremer and W. Colli, *Glycobiology*, 5 (1995) 547–552.
- [3] S. Notermans, G.H. Veeneman, C.W.E.M. van Zuylen, P. Hoogerhout, and J.H. van Boom, *Mol. Immunol.*, 25 (1988) 975–979.
- [4] K. Osumi, M. Enomoto, and H. Sigimura, *Carbohydr. Lett.*, 2 (1996) 35–38.
- [5] (a) A. Arasappan and B. Fraser-Reid, *Tetrahedron Lett.*, 36 (1995) 7967–7970; (b) R. Velty, T. Benvegnu, M. Gelin, E. Privat, and D. Plusquellec, *Carbohydr. Res.*, 299 (1997) 7–14.
- [6] C. Gallo-Rodriguez, O. Varela, and R.M. de Lederkremer, J. Org. Chem., 61 (1996) 1886–1889.
- [7] J. Bogusiak and W. Szeja, Carbohydr. Res., 295 (1996) 235-243.
- [8] J.C. McAuliffe and O. Hindsgaul, J. Org. Chem., 62 (1997) 1234–1239.
- [9] L.A.J.M. Sliedregt, H.J.G. Broxterman, G.A. van der Marel, and J.H.van Boom, *Carbohydr. Lett.*, 1 (1996) 61–68.
- [10] R.M. de Lederkremer, C. Lima, M.I. Ramirez, M.A.J. Ferguson, S.W. Homans, and J. Thomas-Oates, J. Biol. Chem., 266 (1991) 23670–23675.
- [11] (a) S. Mehta and B.M. Pinto, J. Org. Chem., 58 (1993) 3269–3276; (b) S. Mehta and B.M. Pinto, Phenyl Selenoglycosides as Versatile Glycosylating Agents in Oligosaccharide Synthesis and the Chemical Synthesis of Disaccharides Containing Sulfur and Selenium, in S.H. Khan and R.A. O'Neill (Eds.), Modern Methods in Carbohydrate Synthesis, Harwood Academic Publ., Netherlands, 1994, pp. 107–129.
- [12] G.J.F. Chittenden, Carbohydr. Res., 25 (1972) 35-41.
- [13] Y. Kondo, K. Noumi, S. Kitagawa, and S. Hirano, *Carbohydr. Res.*, 123 (1983) 157–159.
- [14] H.M. Zuurmond, P.H. van der Meer, P.A.M. van der Klein, G.A. van der Marel, and J.H. van Boom, J. Carbohydr. Chem., 12 (1993) 1091–1103.
- [15] P.J. Garegg, I. Kvarnstrom, A. Niklasson, G. Niklasson, and S.C.T Svensson, J. Carbohydr. Chem., 12 (1993) 933–953.
- [16] F.-I. Auzanneau and B.M. Pinto, *Bioorg. Med. Chem.*, 4 (1996) 2003–2010.